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CHARACTERIZATION OF AN ANTIGEN EXPRESSED BY HUMAN NATURAL KILLER CELLS[1]

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A monoclonal antibody, anti-N901, was produced by fusing NS-1 myeloma cells with spleen cells of a mouse immunized with human CML cells. This antibody was reactive with a subpopulation of peripheral blood LGL, including the natural killer cells. Monocytes, granulocytes, B cells, T cells (T3+ cells), erythrocytes, and platelets were nonreactive. The N901-positive cells in the peripheral blood were heterogeneous with respect to expression of other cell surface antigens. The majority of N901+ cells co-expressed T11, Mo1, and HNK-1, whereas a smaller percentage expressed T8, Ia, T3, T4, Mo2, or B1 antigens were very uncommon on N901+ cells. The heterogeneity of the N901+ LGL was further investigated by examining the expression of N901 antigen on a series of cloned normal human NK cell lines. N901 antigen was expressed by each of the NK cell lines tested, and by a minority of cloned T cell lines without NK activity. Anti-N901 does not block NK activity and can be used to rapidly purify functional NK cells for further study.

Natural killer (NK)[3] cells are defined by their ability to lyse certain tumor cells *in vitro* in the absence of antibody or previous immunization (1, 2). Although their role in the immune system is not yet clear, it has been postulated that *in vivo* they may mediate natural resistance against tumors, certain viral diseases, and bone marrow allografts (3). Although NK cells constitute only a small fraction (1–5%) of the mononuclear cells of human peripheral blood, the majority appear to be found within a population of cells with distinctive morphologic features termed "large granular lymphocytes" (LGL) (4, 5). These cells have abundant pale cytoplasm with characteristic azurophilic granules and a reniform nucleus. It has been possible to purify human LGL for functional studies (6), and it has been determined that at least some cells share surface antigens with T lymphocytes, including T11, the sheep erythrocyte receptor (7, 8). In addition, the majority of NK cells share an antigen with monocytes and granulocytes that is recognized by the anti-Mo1 (OKM1) (9, 10) monoclonal antibody. It has recently been shown that normal human NK cells can be cloned and expanded *in vitro* while retaining NK function (11, 12). Studies of the surface antigens expressed by these cell lines suggest that NK cells may be very heterogeneous, with some cell lines expressing surface antigens characteristic of T cells whereas others lack T cell antigens or express uncommon phenotypes (12). The identification of surface antigens that are unique to NK cells or subsets of NK cells would greatly facilitate our ability to identify these cells and manipulate them *in vitro*.

Several new monoclonal antibodies have recently been described that react with NK cells (13–17). However, most of these antibodies also react with a substantial number of non-NK cells as well. In this report, we describe the production of a monoclonal antibody, N901, which is highly specific for the subpopulation of LGL cells expressing NK activity, and which appears to be distinct from previously described antibodies.

MATERIALS AND METHODS

Production of monoclonal antibodies. A 6-wk-old female BALB/c mouse (Jackson Laboratory, Bar Harbor, ME) was immunized with cryopreserved leukemic cells from a single patient with the blast phase of chronic myeloid leukemia (CML) (18). These blasts uniformly expressed two surface antigens characteristic of myeloid cells (MY7 and Mo1), and lacked antigens characteristic of lymphocytes, erythrocytes, or megakaryocytes (19). The immunization schedule consisted of an initial intraperitoneal injection of 10^7 cells emulsified in complete Freund's adjuvant (Difco, Detroit, MI), followed by three weekly injections of 10^7 viable cells in phosphate-buffered saline. One month later, 10^7 cells were injected intravenously, followed by splenectomy in 3 days.

Somatic cell hybridization was carried out by the method of Kohler and Milstein (20) with previously described modifications (21). Supernatants from growing hybrid cell clones were tested for reactivity by indirect immunofluorescence on the immunizing cells as described. Clones producing reactive antibody were extensively screened for reactivity with T cell and B cell lines. The clone producing N901 antibody was found to lack reactivity with such cell lines, and was recloned by limiting dilution three times before passage in pristane-primed BALB/c mice as malignant ascites. All experiments were performed with ascites fluid diluted 1:250.

Immunofluorescence assays. Indirect immunofluorescence assays were performed as previously described (18), using 10^6 target cells for each analysis. All media contained 2.5% human AB serum to minimize Fc binding. Fluoresceinated rabbit anti-mouse immunoglobulin (Ig) was obtained from Tago (Burlingame, CA). Fluorescent antibody coated cells were detected on a cytofluorograph (FC200/480DA, Ortho Instruments, Westwood, MA) or a Coulter Epics V (Coulter Electronics, Hialeah, FL). Background fluorescence was measured by using an isotype-identical monoclonal antibody previously shown not to be reactive with test cells.

Dual marker analysis was performed by detecting the first antigen by standard indirect immunofluorescence, using goat anti-mouse Ig coupled to the fluorescent red dye RD2 (Coulter Electronics) as a second step antibody. The cells were then washed two times in medium containing 2% normal mouse serum to block any unbound anti-mouse Ig-binding sites, and then incubated with second antibody directly fluoresceinated for 30 min at 4°C. A minimum of 200 red fluorescent cells were counted in a Zeiss fluorescence microscope, and the percent of these cells expressing detectable fluorescein (green) fluorescence was recorded.

Isolation of human cell fractions. Human granulocytes were prepared from the peripheral blood of healthy volunteer donors by Ficoll-Hypaque density gradient centrifugation. The pellet was diluted 1:1 with Hanks' balanced salt solution containing 0.4% high m.w. dextran (Dextran T500, Pharmacia Fine Chemicals, Piscataway, NJ), and contaminating erythrocytes were removed by unit gravity sedimentation (22). After washing, the granulocyte pellet was treated with 0.17 M NH_4Cl to remove any remaining erythrocytes by hypotonic lysis. Granulocyte preparations were >98% pure by morphologic criteria.

Peripheral blood mononuclear cells were isolated from the interface of Ficoll-Hypaque separated peripheral blood. Monocyte-enriched adherent cells

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[3] Abbreviations used in this paper: NK, natural killer; LGL, large granular lymphocytes; Ig, immunoglobulin; E+, sheep erythrocyte rosette-forming cells; PBL, peripheral blood lymphocytes; PHA, phytohemagglutinin; FBS, fetal bovine serum; CFU-C, myeloid colony-forming cell; AML, acute myeloblastic leukemia; CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; CALLA, common acute lymphoblastic leukemia antigen; AMML, acute myelomonocytic leukemia; APL, acute promyelocytic leukemia; AMoL, acute monocytic leukemia; BM, bone marrow; T, thymus derived.

were obtained by incubating peripheral blood mononuclear cells from defibrinated blood on plastic culture dishes (Falcon Labware, Oxnard, CA) in the presence of 10% pooled human AB serum for 60 min at 37°C. Adherent cells were routinely 60 to 80% monocytes as defined by Wright-Giemsa staining and positive staining for alphanapthylacetate esterase (nonspecific esterase). Thymus-derived (T) lymphocytes were isolated by rosetting with 5% sheep erythrocytes treated with 2-aminoethylisothiouronium hydrobromide (Sigma Chemicals, St. Louis, MO) (23). Purity of these preparations was also determined by monoclonal antibodies specific for monocytes (anti-Mo2) and T cells (anti-T3) (9, 24). Platelets were prepared from heparinized blood by low speed centrifugation to remove cells, and purity was assessed by microscopic examination. In all cases, reactivity is expressed as the percentage of a cell population more fluorescent than control cells in an indirect immunofluorescence assay. The control antibody was a murine monoclonal IgG or IgM previously shown to be nonreactive with test cells. Ascites diluted 1:100 to 1:500 was used in all cases.

Human bone marrow was obtained from healthy volunteers by aspiration into preservative-free, heparin-containing syringes. Erythrocytes and mature neutrophils were removed by Ficoll-Hypaque sedimentation.

Human thymocytes were obtained from thymuses removed for surgical reasons in children undergoing cardiac surgery. Human splenocytes were obtained from spleens surgically removed because of trauma. Peripheral blood cells rosetting with sheep erythrocytes (E+) were "activated" by exposure to lectin (phytohemagglutinin 2 μg/ml), with aliquots removed for phenotypic analysis on day 0, day 3, and day 7.

Cell separation. Fluorescent cells were separated from nonfluorescent cells by fluorescence-activated cell sorting with a Coulter Epics V (18). Background fluorescence was determined with a nonreactive, isotype-identical monoclonal antibody, and cells fluorescent above background were sorted into 50% fetal bovine serum (FBS) at a rate of 3000 cells/sec. Sorted cells were then washed twice and suspended in RPMI 1640 medium containing 5% FBS for functional assays or preparation of cytocentrifuge smears.

NK cell assay. NK cell activity was determined in a 4-hr assay with the use of ^{51}Cr-labeled K562 and effector to target ratios of 50:1 to 6:1 (11).

Monoclonal antibodies. Anti-I2 (anti-Ia), -T3, -T8, -T10, -T11, -Mo1, -Mo2, and -B1 have been previously described (9, 24–26). T3 is a pan-T cell antigen, T11 is the E rosette receptor, T8 is an antigen of cytotoxic/suppressor T cells, and T10 an antigen of early thymocytes, certain activated lymphocytes, and hematopoietic precursor cells. Mo1 and Mo2 are expressed by monocytes, and Mo1 is additionally expressed by granulocytes and some non-T, non-B (null) peripheral blood lymphocytes (PBL). Mo1 and OKM1 identify identical structures. B1 is expressed by B cells. HNK-1 (Leu7, Becton Dickinson, Sunnyvale, CA) reacts with human NK cells (13). All antibodies were used at a dilution determined to be in antibody excess.

Cloned human NK cell lines. LGL were cloned by limiting dilution, stimulated by phytohemagglutinin, autologous or allogeneic Epstein-Barr virus-transformed B cells, and expanded in medium containing lymphocyte-conditioned medium as previously described (11, 12). Cell lines were tested for NK activity as described above, and phenotype analysis was performed by indirect immunofluorescence. The phenotypic and functional heterogeneity of these cell lines have been previously described (12). Several clones of T11+ cells that did not have NK activity were similarly prepared and analyzed for expression of N901 antigen.

CFU-C assay. The myeloid colony-forming cell (CFU-C) was assayed in agar by using a feeder layer of peripheral blood leukocytes as a source of colony-stimulating factors as previously described (18). Bone marrow cells were separated into N901 positive and negative cell fractions by an immune rosette technique previously described in detail (27) before CFU-C assay. Colonies were counted in an inverted microscope on day 7 and day 14.

Human leukemia cells. Tumor cells were obtained from the peripheral blood or bone marrow of 59 patients with acute myeloblastic leukemia (AML), 21 patients with blast phase chronic myeloid leukemia (CML-BC), 67 patients with acute lymphoblastic leukemia (ALL) and 27 patients with chronic lymphocytic leukemia (CLL). Standard morphologic and cytochemical criteria were used to establish the diagnosis in each case. In addition, surface marker analysis with a panel of monoclonal antibodies recognizing appropriate differentiation antigens of myeloid, erythroid, B lymphoid, and T lymphoid cells were used to confirm the clinical diagnosis (19). AML patients were subgrouped, using morphology and cytochemical staining as undifferentiated AML, acute myelomonocytic leukemia (AMML), acute monocytic leukemia (AMoL), and acute promyelocytic leukemia (APL). The J5 monoclonal antibody was used to separate ALL samples into common acute lymphoblastic leukemia antigen (CALLA) positive and negative groups (28). Some bone marrow samples were thawed in the presence of deoxyribonuclease I, 10 μg/ml (Millipore Corp, Freehold, NJ) to minimize agglutination of cells. Analysis of leukemic samples on the cytofluorograph was performed as previously described (18). A positive reaction was arbitrarily taken as greater than 25% of test cells staining more intensely than with control antibody. All leukemic samples contained >80% tumor cells, and generally >90%.

Human cell lines. The myeloid cell line KG-1 was supplied by Dr. David Golde, University of California, Los Angeles, School of Medicine. All other cell lines were provided by Dr. Herbert Lazarus, Sidney Farber Cancer Institute.

RESULTS

Specificity of N901 antibody. The expression of N901 antigen on peripheral blood (PBL) and bone marrow (BM) cells is shown in Table I. Approximately 7% of peripheral blood mononuclear cells (MNC) reacted with anti-N901 with all of these cells having the light scatter profile of lymphocytes. When leukocyte subpopulations were purified, significant reactivity was detected in the E+ cell fraction (7.8 ± 4.8%) and the null cell fraction (PBL MNC depleted of E+ cells and adherent cells, 47.5 ± 18.5%) (Fig. 1). Granulocytes and B lymphocyte fractions showed no reactivity whereas monocytes showed minimal increased fluorescence

TABLE I

Expression of N901 antigen on normal peripheral blood and bone marrow cells

Cell Fraction*	No.	% Positive Cells
I. Peripheral blood		
Mononuclear cells	20	6.7 ± 2.9
Granulocytes	6	0.6 ± 0.4
Monocytes	5	4.2 ± 3.1
E+ lymphocytes	5	7.8 ± 4.8
E− lymphocytes	6	47.5 ± 18.5
B lymphocytes	2	2.9 ± 1.0
Erythrocytes	4	0.1 ± 0.1
Platelets	2	0.1 ± 0.1
PHA-T cells, day 3	3	4.7 ± 1.0
PHA-T cells, day 7	3	1.7 ± 1.5
II. Bone marrow		
Mononuclear cells	11	4.2 ± 3.2
CFU-C	2	<1
III. Thymocytes	3	1.0 ± 1.0
Tonsil cells	4	1.0 ± 1.0
Spleen cells	5	1.0 ± 1.0

*Mononuclear cells were prepared by Ficoll-Hypaque sedimentation. E+, sheep erythrocyte-rosetted cells; PHA-T cells, E+ cells stimulated with phytohemagglutinin; CFU-C, myeloid colony-forming unit.

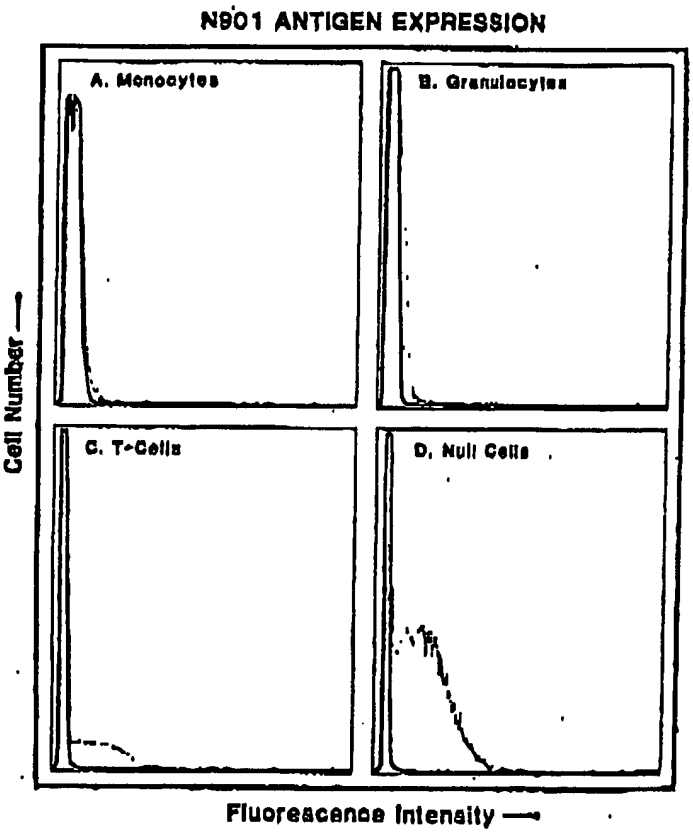

Figure 1. Monocytes, granulocytes, T cells or "null" cells were purified as described in *Materials and Methods* and tested for N901 antigen expression by an indirect immunofluorescence assay. In each panel, the dark line indicates control antibody fluorescence and the light line indicates anti-N901 fluorescence.

over background (4.2 ± 3.1%). When analyzed by fluorescence microscopy, purified monocytes were not detectably fluorescent. A small number of N901 fluorescent cells could be seen in the E+ cell fraction, although fluorescence was dim compared with control antibodies anti-T3 and anti-T8. There was no detectable N901 on erythrocytes or platelets. PHA activation of T cells did not increase N901 expression. However, culture of PHA-activated T cells with conditioned medium containing T cell growth factor (11) for an additional 7 days resulted in expression of N901 on a fraction (20%) of cells. N901 was expressed by 4.2 ± 3.2% of BM MNC that were nongranulated by phase microscopy and did not include the myeloid colony-forming cell, CFU-C. N901 antibody was determined to be an IgG1 by Ouchterlony immunodiffusion assay in agar, and was not lytic against the immunizing cells by trypan blue exclusion.

The following cell lines were found not to express N901 antigen by indirect immunofluorescence: (myeloid, erythroid) HL60, HL60-blast, U937, K562; (B cell) Laz 388, Laz 471, Laz 509, Laz 156, SB; (T cell) CEM, HSB, Molt-4; (lymphoblastic leukemia, lymphoma) Laz 221, Nalm-1, Daudi, Raji. The KG-1 (myeloblast) cell line was positive.

Characterization of the N901+ lymphocyte subset. N901+ cells were separated by cell sorting from PBL MNC or E− cells depleted of adherent cells (Fig. 2) and cytocentrifuge smears of each fraction stained with Wright-Giemsa stain. The percent of large granular lymphocytes (LGL), lymphocytes and monocytes in each fraction was determined (Table II). As shown, the N901+ cell fraction was primarily composed of LGL with very small numbers of small lymphocytes and monocytes. In contrast, the 901− fraction had been substantially, but not completely, depleted of LGL. These results suggested that the N901+ peripheral blood cells are a subset of LGL. Because of the known association of NK activity with human LGL, NK activity was assayed in N901+ and N901− cell fractions. Fig. 3 represents one of seven similar experiments. NK activity was enriched in the N901+ cell fraction, and depleted in the N901-cell fraction. Virtually identical results were obtained with experiments using whole MNC or the E− cell fraction. Peripheral blood cells could also be readily separated into N901+ and N901− fractions, using immune rosettes (27). NK activity was similarly enriched in the N901+ cell fraction was depleted from the N901− cell fraction in multiple experiments starting with whole MNC, E− cells or E+ cells. These results suggest that the majority of NK cells are contained within the N901+ cell fraction despite their heterogeneity in expression of other cell surface antigens. There was no evidence that anti-N901 blocked cytotoxicity against K562, Daudi, or Molt-4 target cells when added to unseparated peripheral blood effector cells.

Cell surface phenotype of N901+ cells. In order to further investigate the surface antigens of NK cells, the phenotype of N901+ cells in the peripheral blood was determined by two-color immunofluorescence analysis. Blood MNC were first incubated with anti-N901 followed by RD2-conjugated anti-mouse immunoglobulin (red fluorescence), and then incubated with directly fluoresceinated second antibody. Table III shows the results of

FACS SEPARATION OF 901⁺ CELLS FROM E⁻ PBL

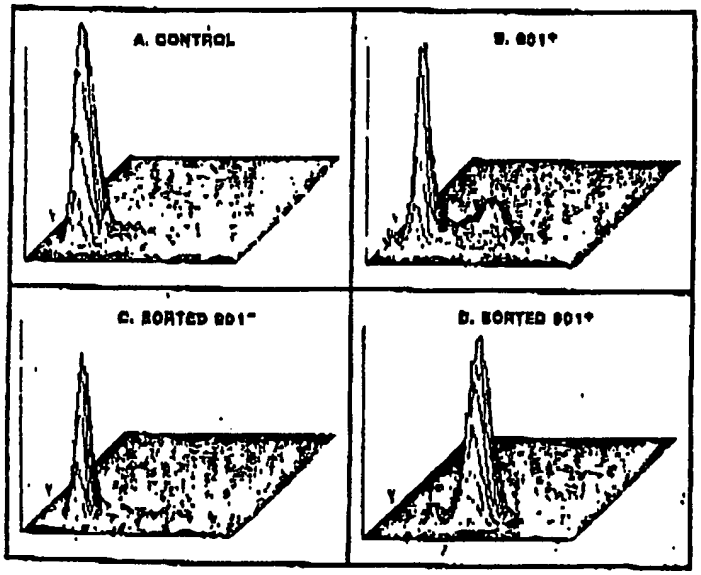

Figure 2. E− PBL mononuclear cells were separated into N901+ and N901− cell fractions by fluorescence activated cell sorting using a Coulter Epics V cell sorter. Fluorescence is shown on the horizontal axis, cell number (10,000 cells counted) on the vertical axis, and forward angle light scatter (a measure of cell size) on the third axis. Reanalysis of the sorted cells (*panels C and D*) showed that the purity of the sorted cell fractions was >97%.

TABLE II

Morphology of the N901+ cell fraction

Experiment	Cell Fraction[a]	% of Total[b]	Morphology[c]				
			Small Lymphocytes	LGL	Monocytes	Basophils	Other
			%				
1. Nonadherent PBL	Unseparated	—	80	12	6	1	1
	N901+	17.6	3.5	95	1	0.5	0
	N901−	82.4	82	11	5	1	1
2. E rosette −, nonadherent PBL	Unseparated	—	45	39	12	2	2
	N901+	43.5	6	92	1	0.5	0.5
	N901−	56.5	62	11	21	4	2

[a] Cells were separated into N901+ and N901− fractions by fluorescence-activated cell sorting.
[b] Percent of total cells recovered after sorting.
[c] Determined after staining cytocentrifuge smears with Wright-Giemsa stain. LGL, large granular lymphocytes; Other, includes neutrophils, eosinophils, and unidentifiable cells.

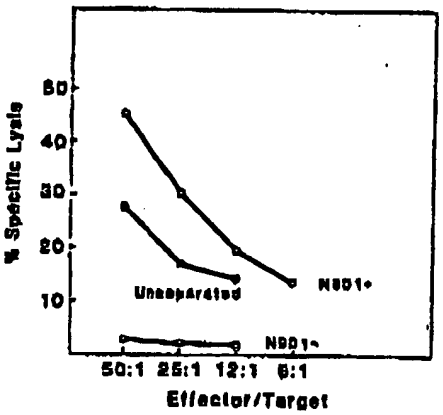

Figure 3. Whole PBL MNC were separated into N901+ and N901− cell fractions by cell sorting, and NK activity was determined by using K562 target cells at various effector to target cell ratios.

TABLE III

Cell surface antigens expressed by N901+ cells

Surface Antigen	Defined Cell Reactivity	% of Total Cells Positive[a]	% of N901 Cells Positive
N901	LGL	7 ± 2	—
T3	T cells	72 ± 6	2 ± 1
T4	Helper T cells	48 ± 7	1 ± 1
T8	Cytotoxic/suppressor T	27 ± 5	27 ± 6
T11	E rosette receptor	82 ± 4	74 ± 10
I2	HLA-DR (Ia)	15 ± 4	2 ± 2
Mo1	Monocytes, granulocytes, NK	14 ± 4	80 ± 16
Mo2	Monocytes	6 ± 1	0
B1	B cells	4 ± 2	0
Leu7	LGL (HNK-1)	14 ± 5	85 ± 14

[a] Mononuclear cells of three healthy donors were tested. Mean ± SD.

such an analysis in three subjects. The majority of the N901+ cells were T11+, Mo1+, and Leu7+. A subset was T8+, although the T8 fluorescence on N901+ cells was distinctly decreased, compared to T8 fluorescence on N901− cells. Ia, T3, T4, Mo2, and B1 were uncommon or negative as previously described (11).

Expression of N901 on cloned human NK cell lines. In order to further characterize the expression of N901 antigen on NK cells, a series of cloned NK cell lines and cloned T cell lines were studied (Table IV). N901 antigen was expressed by all of the cells of four cloned lines of cells (JT1, JT3, JT9, JT10) with strong NK activity. As previously described, these cell lines are composed of LGL, but express heterogeneous surface antigen phenotypes (12). JT1, for example, expresses no T cell-associated antigens, whereas JT9 has the phenotype of a cytotoxic/suppressor T cell (T3+, T8+, T4−). In contrast, of six cloned T11+ cell lines lacking NK activity, only one (NONK1) showed strong expresson of N901. However, several other non-NK T cell lines (NONK3, NONK5) had a small percentage of cells (<20%) which expressed N901. Thus, although freshly isolated resting and PHA-activated T3+ cells do not appear to express N901 antigen (Tables I and III), some T cell lines lacking NK activity may express at least low levels of the antigen.

Expression of N901 antigen on hematopoietic malignancies. The immunizing cell type used in the generation of anti-N901 was CML blast crisis. It was thus of interest to determine the expression of N901 in hematopoietic malignancies. As shown in Table V, the blasts of 15% of 59 AML patients expressed N901. N901+ AML cells were brightly fluorescent and, in positive patients, generally all the leukemic cells were fluorescent. Of 21 CML blast crisis patients studied, 3 patients' cells were positive, all with myeloid blast crisis. Of 67 ALL cases, 2 showed weak fluorescence with anti-N901. It was not determined if the positive cells were definitely leukemic, or were residual normal cells. None of 21 cases of CLL were N901+. Thus, the principle expression of N901 antigen in leukemias is in myeloid leukemia. Nonhematopoietic malignancies or normal tissues have not been investigated.

TABLE IV

Expression of N901 antigen on cloned human NK and T cell lines

Cloned Cell Line	Phenotype[a]	NK Activity	N901[a]
JT1	T3−, T4−, T8−, T11−, Mol±, HNK−1−	+	+
JT3	T3−, T4−, T8+, T11+, Mol+, HNK−1−	+	+
JT9	T3+, T4−, T8+, T11+, Mol−, HNK−1+	+	+
JT10	T3+, T4−, T8+, T11+	+	+
NONK1	T3+, T8+, T11+	−	+
NONK2	T11+	−	−
NONK3	T11+	−	+/−
NONK4	T11+	−	−
NONK5	T11+	−	+/−
NONK6	T11+	−	−

[a] Antigen expression was determined by indirect immunofluorescence: +, bright fluorescence of all cells; +/−, dim fluorescence of all or some cells; −, no fluorescence of cells. All antigens that were tested on a given clone are shown.

TABLE V

Expression of N901 antigen on leukemias

Leukemia	No. Tested	No. Positive[a]
Acute myeloblastic leukemia (AML)		
AML (M1, M2)	36	6
APL (M3)	3	0
AMML, AMoL (M4, M5)	20	3
Chronic myeloid leukemia (CML)		
Blast crisis (CML-BC)		
Myeloid	15	3
Lymphoid	5	0
Megakaryoblast	1	0
Acute lymphoblastic leukemia (ALL)		
Non-T, CALLA+	44	2
Non-T, CALLA−	13	0
T-ALL	7	0
B-ALL	3	
Chronic lymphocytic leukemia (CLL)		
B-CLL	23	0
T-CLL	4	0

[a] Determined by an indirect immunofluorescence assay and fluorescence-activated cell sorter analysis. Samples were considered positive if greater than 25% of cells were fluorescent compared to control monoclonal antibody. All patients who were positive had greater than 75% brightly fluorescent cells, except the two ALL patients who had 20 to 50% weakly fluorescent cells.

DISCUSSION

This study describes the initial characterization of a monoclonal antibody, anti-N901, expressed by the majority of human NK cells. Unlike several previously described antibodies, N901 is not expressed by granulocytes, monocytes, T lymphocytes (T3+ cells), or PHA-activated lymphocytes, and thus appears to represent a distinct antigen (13, 14, 16, 17). Morphologically, the N901+ cells are a homogeneous population of LGL, although not all cells with this morphology are N901+. This suggests that NK-active cells are a subset of LGL, and that a substantial fraction of LGL may be cells of a separate lineage (T3+ cells, for example). When N901+ cells are isolated by FACS, only a very small amount of residual NK activity could be detected in the N901− fraction.

It has been previously recognized that the surface antigens of NK cells are heterogeneous (8, 10–12). The majority of NK cells have been found to express Fc receptors, a low affinity receptor for sheep erythrocytes, the Mo1 antigen, T10 antigen, and Leu7 (3, 7–9, 11, 13). Further, NK cells have been reported to lack Ia, T3, and monocyte antigen Mo2 (11). The surface antigen phenotype of N901+ peripheral blood cells was therefore determined in samples of blood from three donors. The majority of N901+ cells expressed Mo1, T11, and Leu7. A small population (27%) expressed T8, whereas T3, T4, Ia, B1, and Mo2 were uncommon or negative (Table III). Recent studies by Abo and colleagues (29) have shown that the Leu7+ population can be divided into two subgroups: Leu7+T3+M1− (30.6%) and Leu7+T3−M1+ (62.1%). The Leu7+T3−M1+ subgroup has substantially more NK activity than the Leu7+T3+ fraction. Further, the Leu7+T3− fraction had more cytoplasmic granules than did the Leu7+T3+ fraction. Both fractions could be readily distinguished from Leu7− cells by morphology and NK activity. The results presented here suggest that N901 antigen would be preferentially expressed on the Leu7+T3−M1+ cell fraction.

These results demonstrate that the N901+ cells in the peripheral blood are heterogeneous with respect to expression of other cell surface antigens, including T8 and Mo1. Also of note is that a very small number of peripheral blood cells coexpress T3 and N901. In order to investigate this heterogeneity further, the expression of N901 by cloned cell lines with and without NK activity was investigated (Table IV). All of the NK active clones were brightly fluorescent with anti-N901. Also, one non-NK T cell was brightly fluorescent and two others were dimly fluorescent. The NK clones were representative of a variety of cell types (Table IV) and the expression of N901 antigen by these diverse cell types suggests that N901 antigen expression by NK active cells is not confined to a single phenotype. The expression of N901 antigen by non-NK T cell clones is of interest. This is consistent with the observation that in freshly isolated peripheral blood, N901 was detected on an occasional T3+ cell (Table III). It is possible that the non-NK T cell clones that express N901 are derived from this cell.

The expression of N901 antigen on hematopoietic malignancies is of interest. Fifteen percent of AML patients expressed N901 antigen and were usually brightly fluorescent, in contrast to the moderate fluorescence of N901+ LGL. Of note, lymphoid malignancies were essentially negative, although two of 67 ALL's showed weak staining.

The pattern of expression of N901 distinguishes this antigen from other previously described LGL cell antigens. The lack of reactivity with thymocytes, activated T cells, and T cell malignancies distinguishes N901 from T10. The lack of reactivity with granulocytes distinguishes N901 from B73.1, VEP, 13, and Mo1. Further, the relative restriction of N901 antigen to LGL cells further suggests that this antibody may be useful for the isolation of these cells. In addition to fluorescence-activated cell sorting (Table II), it has been possible to enrich for N901+ cells (and NK cells) by using an immune rosette technique previously described (27). This technique allows for the rapid preparation of large numbers of cells sharing a defined antigen. Using these cell separation techniques, it should be possible to further isolate the NK cells within the N901+ cell fraction, and to investigate heterogeneity of cell function within the N901+ cell fraction.

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